Ion chromatography

Rapid and sensitive determination of biofuel sugars by ion chromatography

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Abstract

Purpose: To develop a fast IC method for the determination of sugars in biofuel samples using a Thermo Scientific™ Dionex™ CarboPac™ SA10-4µm Column with electrolytically generated eluent and a high-pressure ion chromatography (HPIC) system with electrochemical detection.

Methods: Biofuel sugars are ionized in a strong base, separated by high-performance anion-exchange (HPAE) chromatography, and detected by pulsed amperometric detection (PAD). The analysis is facilitated by the Thermo Scientific™ Dionex™ Integrion™ HPIC™ system.

Results: Using this method, carbohydrates present in 10 individual field biofuel samples were quantified. The results for the method linearity, precision, accuracy, and robustness are presented.

Introduction

Lignocellulosic biomass is a key renewable source for alternative energy generation.[1–2] As new sources of biomass are explored, approaches to maximize energy generation are becoming important for biofuel producers. Monitoring the release of 5- and 6-carbon sugars, which can be fermented into biofuels like ethanol[3] and biodiesel[4], is required for evaluating the overall efficiency of biomass-to-biofuel conversion and is directly related to target biofuel yield and process economics.[5] Hence, the determination of carbohydrates in hydrolysates derived from lignocellulosic biomass is a crucial step in biofuel production, although the development of robust analytical methods remains a challenge. Currently used methods suffer from several drawbacks such as low throughput, poor analyte resolution, and non applicability to a wider set of biomass samples.[6]

This work updates the column used in AN1161[7] with a shorter 4 × 150 mm format column. The combination of the smaller resin particle size of the Dionex CarboPac SA10-4µm column and the shorter column used here results in separation of eight common sugars in less than six minutes. This is significant time savings as compared to the eight-minute runtime achieved in AN1089.[8] The shorter run time allows for faster sample turnaround times and reduced eluent consumption, thereby improving the overall process economics. Results for method linearity, accuracy, and robustness are presented here. Moreover, using this method, carbohydrates present in 10 individual field biofuel samples were quantified.

Materials and methods

Sample preparation

The biomass hydrolysate samples tested in this study were derived from corn stover, switchgrass, and/or energy cane. These biomass samples were pretreated either individually or as mixtures (mixed feedstocks) with any one of the reagents: acid, alkali, or ionic liquids. The pretreatments were followed by enzymatic hydrolysis with CellicR Ctec2 and CellicR Htec2 (Novozymes, CA) to yield near-complete hydrolysis of biomass oligosaccharides. All biomass hydrolysate samples were diluted 20-fold with deionized (DI) water before chromatography.

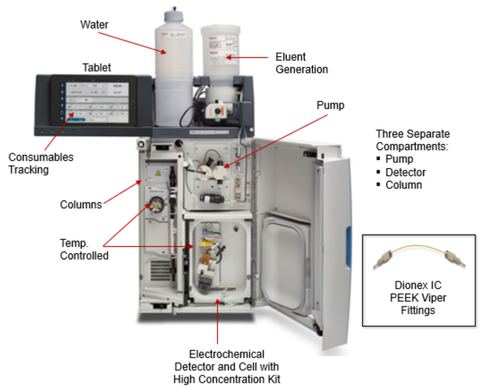
Chromatography

See chromatograms for conditions.

Instruments

Dionex Integrion HPIC system, RFIC model (Figure 1) configured for electrochemical detection, including eluent generation and Thermo Scientific™ Dionex™ IC PEEK Viper™ Fittings (Figure 1, bottom right). The flow diagram is shown in Figure 2.

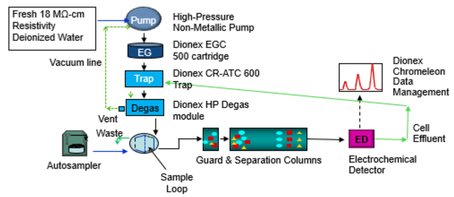
Figure 1. Integrion HPIC System Configured for Electrochemical Detection.



Data Analysis

Thermo Scientific™ Chromeleon CDS™ software, version 7.2 SR4.

Figure 2. Flow diagram for the Dionex Integrion HPIC Reagent-Free system configured for ED detection.



In PAD using the four-potential waveform, the disposable working electrode is pulsed through the different potentials at set times, completing two cycles within one second (Figure 3). This waveform is optimized to provide a clean, stable gold layer in preparation for detection of the next eluting peak.

Results

Separation

A representative chromatogram of a standard mix containing eight of the common biofuel sugars each at 0.6 g/L concentration each was obtained using a Dionex CarboPac SA10-4µm 4 × 150 mm column is shown in Figure 2. Fucose was used as an internal standard, All the peaks were resolved and the separation was completed within six minutes. The smaller particle size and shorter column format result in a faster run time than the previous work.[7]

Linearity

Using this method, peak area calibration curves more than two orders of magnitude wide, between 0.005 and 2 g/L, were generated for all eight sugars. This calibration range is suitable for handling samples without the need for significant dilution, which in turn will avoid dilution errors. The results from the linear curve fitting of the data are included in Table 1.

Figure 2. Separation of biofuel sugars on the Dionex CarboPac SA10-4µm 4 x 150 mm column.

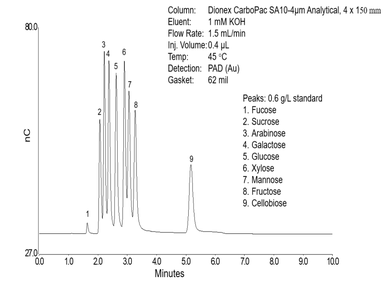
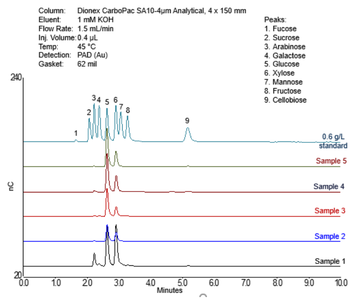


Figure 3. Separation of biofuel sugars on the Dionex CarboPac SA10-4µm 4 x 150 mm column.



Accuracy

Method accuracy was evaluated by first quantifying the sugars present in biomass hydrolysate samples. To determine method accuracy, ten field biomass hydrolysate samples were procured. The samples were collected at various stages of biomass processing and conversion of lignocellulosic biomass to ethanol. From this set, five samples were randomly selected for recovery studies to determine method accuracy. Representative chromatograms for the selected five biofuel samples are shown in Figure 3. The major sugars present in these samples are glucose and xylose. All the biofuel sugars that are present above the lowest calibration standard were spiked with 50-150% of the original amount found in the sample. The original amounts present, spiked amounts, and percent spike recoveries for a representative sample are shown in Figure 4. For the other four samples studied here, excellent recoveries were obtained for all biomass sugars that were spiked (not shown).

Table 1. Calibration (at 11 levels between 0.006 to 2 g/L of each of the eight common biofuel sugars) and precision data (n=3)

Peak Name	Ret.Time (min)	Resolution	Concentration Range (g/L)	Coeff.of Determination
Sucrose	2.13	1.4	0.006-2	1.000
Arabinose	2.28	1.4	0.006-06	1.000
Galactose	2.45	2.0	0.006-06	1.000
Glucose	2.71	2.1	0.006-1	0.999
Xylose	3.00	1.2	0.006-06	0.999
Mannose	3.17	1.5	0.006-06	0.999
Fructose	3.40	10	0.006-1	0.999
Cellobiose	5.48	-	0.006-2	0.999

Robustness

First, variation in method performance was monitored over a 180 - injection sequence run of the biofuel samples. Changes in retention time and peak area were measured over all the samples by injecting at frequent intervals a standard containing 0.6 g/L of all eight biofuel sugars. This resulted in 21 standard injections during the total sequence run time of ~33 h. Figures 5A and 5B show retention time and peak area trending of all the 21 standard injections. Maximum retention time and peak area change values observed were 0.8% and 1.8% both for xylose. Hence there was no significant trending of either peak area or retention time during this experiment. This result indicates that the method is able to withstand sample matrix effects in a typical sequence run.

Figure 5A. Retention time stability to matrix effects.

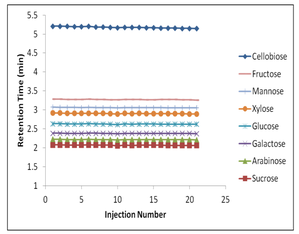
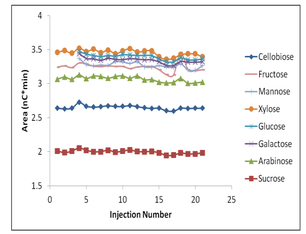


Figure 5B. Peak area stability to sample matrix effects.



Conclusions

- The method can separate all eight biomass sugars within 6 minutes
- The response data for all biomass sugars studied here show excellent correlation with concentration
- Spike recovery studies demonstrate that the method is accurate.
- Method robustness: stable peak retention times and areas over 180 sample injections

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